**SYNOPSIS OF PH.D TOPIC**

**Entitled**

# GENOMIC, MORPHOLOGICAL DIVERSITY OF FRESHWATER FISH IN RELATION TO PHYSICO-CHEMICAL PARAMETERS OF MANJRA RIVER.

**Submitted to**

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**Title of Ph.D. Work**

**Genomic, Morphological Diversity of Freshwater Fish in Relation to Physico-chemical Parameters of Manjra River.**

## Introduction:

The variety of plants, animals and microorganisms on Earth, along with the tremendous diversity of genes in these species and the high variety of ecosystems on the globe are all constitutive parts of what is called "biodiversity". It is globally recognized that the conservation of nature and of its biological diversity should represent one of the priorities of the current human society. An important number of factors, sometimes interconnected, like climate change, pollution, anthropic activities destroying habitats for agriculture or logging, excessive exploitation of species with economic importance, hunting or poaching, represent serious treats for biodiversity.

Mittermeier RA et.al have shown that, India ranks ninth in the list of countries with the highest levels of biodiversity in the world in terms of mega biodiversity in freshwater [1]. 11.72% of the world's fish population is in India. Species, 23.96% of genera, 57% of families, and 80% of all fish species worldwide are represented [2] Among the 2200 the cold freshwater regime, 73 (3.32%) and 544 (24.73%), respectively, of the species that have been listed so far.143 (6.50%) belong to the warm freshwater domain, 1440 (65.45%) to the brackish waters, and aquatic ecosystem

Globally, there are roughly 450 families of freshwater fish. There are about 40 represented in India. (Species of warm freshwater). About 25 of these families contain commercially important species. There are around 544 endemic species in warm water [3, 4]. The earth is a planet; there are four parts to the ecosphere. The hydrosphere is one of them. In the biosphere, water is a crucial element that can be found in gaseous, liquid, and solid forms states. On the entire planet, there are thought to be 1350 km2 of water, of which 97.4% is liquid is found in oceans by volume. The biological production and physico- chemical composition of aquatic body are directly correlated to the status that has the potential for fisheries resources and tropic status [5]. Aquatic life Physical and chemical properties of the environment play a significant role in determining the biota's ability to create numerous adaptations that enhance long-term productivity and control lake metabolism [6].

In India biodiversity among animals are like 7.6% of all mammalian, 12.6% of all avian, 6.2% of all reptilian, 4.4% of all amphibian, 11.7% of all Pisces group, and 6.0% of all flowering plant species [7]. Sampling, identifying and arranging a species systematically are the pioneer work toward protection of biodiversity. Thus, it is a duty of researcher to precisely identify a species for the purpose of conservation and sustainable use.

An understanding of the taxonomy and systematics of fish species is a pre-requisite for sustainable management of genetic resources. DNA barcoding enhances the prospects for species-level identifications globally using a standardized and authenticated DNA basedapproach [8]. The application of cytochrome c oxidase I (COI) gene for species identification in fish triggered the international initiative for barcoding all fishes [9, 10].

DNA barcoding provides an accurate and automated species identification system through the use of molecular tags based on short and standardized mitochondrial genes [11].

Present investigation will provide molecular database for fish diversity of Manjra River for future researchers. No attempt has been noted previously on Manjra River.

**Review of Literature:**

The knowledge of fish diversity is essential for adopting proper conservation strategies. Accurate identification of fish is the major task for a taxonomist to develop appropriate conservation management. Every fish species has individual genetic distances depending upon the environmental stress and water quality which play an important role in its minor morphometric variations. Many species have been considered cryptic that mean they are morphologically indistinguishable so that their status remains argumentative. [12].

Consequently, classification and identification of fishes through classical taxonomy have various barriers and limitations.

Nowadays, many researchers use morphological analysis and DNA barcoding work collaboratively for species identification. DNA barcoding as a molecular approach provides an effective tool in fish identification. After the establishment of global barcode database for fishes, even inexpert or less experienced user by accessing DNA sequences will be able to distinguish all fish species and the identification sustained by it could be used to evaluate fish biodiversity, monitor fish conservation and manage fisheries. FISH-BOL (the Fish barcode of life campaign), international research collaboration, collected DNA barcode records for the entire world's fishes with the aim to develop COI gene sequences library [13].

In India, DNA barcoding is in the infancy period and should be focused on in the sampling of marine and freshwater species. Other than the COI barcode marker (mitochondrial gene), the effort should be targeted to develop a nuclear barcode because sometime COI fails to distinguish fishes formed through introgressive hybridization. Thus, this may help to develop fish DNA barcoding more easily as well as quickly. But the challenge is to find out 600-1000 bp long nuclear coding region undisrupted by introns, with fast rates of evolution [14]. However, researches from interdisciplinary sciences are desirable in this direction to fulfill that goal.

**Objectives of Research:**

1. To study morphological fish diversity: Identification by Day Volume
2. To study of fish diversity population by Weiner index (H), Simpson Dominance index (D), Simpson index of diversity (1-D).
3. To study Physico-chemical parameter in relation to fish diversity suggested by APHA.
4. To study genomic fish diversity by DNA Barcoding.

**Materials and Methods:**

**2. About study site:**

Manjra, is major river in India with potential fish production . It rises from Gaurwadi in Balaghat ranges of hills near Ahmednagar district at an altitude of 823 metres (2,700 ft) .It has a total catchment area of 30,844 Sq / Km (3,084,400 ha) and flows in West to East direction. During its total course of 724 Km, Manjra river passes through the states of Maharashta, Karnataka and Telangana. Manjra is not a transboundary river. It originates and ends within the Indian borders. It empties in Godavari river. Manjira is an intermittent river. Its flow significantly reduces or completely stops during certain parts of the year. Major left bank tributaries of manjra include Gharni, Wakee, Rena, Tiru and Lendi. On the right-bank, Manjira receives tributaries named Terna and Tawrja. Authorities have built dams and water reservoirs on manjira and its tributaries. These dams/reservoirs include Nizam Sagar Dam and Singur Dam.

**3. Sampling of Fishes:**

The tissue and voucher specimens will be collect from different spots of Manjra river, Latur district. Species identification and nomenclature followed the FAO Fish Identification Sheets. Approximately 100 mg of white muscle tissue and fin clips from each species will preserve in 95% ethanol until used.

**4.** Physico-chemical parameter will be study followed by standard method suggested by APHA.

**5. DNA barcoding**:

The extraction of the DNA protocol will be varying in laboratory, but with fresh tissue from fish, almost all extraction protocols will yield amplifiable DNA. Significant value can be added to a specimen by performing archival quality DNA extractions. Further Amplification of fish DNA via PCR is rarely problematic, and follows standard protocols. The primers will be robust, amplifying almost all taxa tested to date. The same is true for DNA sequencing. Finally, as much of fish biodiversity is known, we have been able to generate a reference sequence. This reference with degenerate bases to represent variation among fish taxa eases the alignment and analysis of fish data.

**6.** All the sample further will use for study of genetic diversity and phylogenic study.

**International and National Status and Significance of Study:**
**International Status:**

The concept of DNA barcoding as a genomic identification system was developed by Paul Hebert and it is a well-accepted taxonomic method to facilitate species identification even by a non-specialist.

In DNA barcoding, a short standardized nucleotide sequence of DNA is used for the identification of fish. Generally, cytochrome oxidase subunit 1 (cox 1), which is a mitochondrial DNA gene used as a global bio-identification system for an animal[15].

The process of DNA barcoding includes the following steps: DNA extraction i.e. isolation of DNA from the sample, PCR amplification of the target DNA barcode region, DNA sequence of PCR products and analysis. The basic principle behind DNA barcoding is that the interspecies variations are more than intraspecies variation, which helps to differentiate the species using short standardized nucleotide sequences. The main objectiveof DNA barcoding emphasis on the gathering of reference libraries of barcode sequences for known species so that after assembly a consistent molecular tool is developed for species identification.

DNA barcoding has been effectively used for both marines and freshwater species.

Ward and Robert D. in 2005 had shown that, first of all discriminated Australian marine fish through DNA barcoding. They generated 754 barcodes for 207 species. They sequenced multiple specimens of 3 species of Chimaerids, 61 species of sharks and ray, and143 species of teleost for the barcode region of cox1 [16]. Moura et al. (2008) attempted to test the competence of the DNA barcode approach to discriminate deepwater shark species: Centrophorus squamosus, Centrophorus granulosus, Centroscymnus coelolepis and Centroscymnus owstoni and their study revealed a low level of haplotypic and genetic diversities [17].

Holmes et al. (2009) ) identified shark species from dried fins in Australian water. They examined 211 left pectoral fins, 193 fins out of them provided a chondrichthyan sequence which when matched with reference specimen in a DNA barcode database identified 27 species of shark and rays among them, twenty species were sharks while seven were rays. They found that Carcharahinus dussumieri was the most abundant fish [18]. Additionally, Steinke et al. (2009) constructed a DNA barcode reference sequence library for introduced ornamental fish species in North America [19].

The mega diverse neotropical freshwater fish fauna was examined by Pereira et al. (2013) using DNA barcoding. They analyzed 254 species, out of which 252 were correctly identified by DNA barcode sequences (99.2%). They tested the competence of the COI gene in identifying freshwater fish fauna from the neotropical region. Genetic divergence, measured by using K2P, was 0.3% and 6.8% for intra and inters specific diversity, respectively [20].

**National Status:**

Remarkably, in India, many researchers examined Ichthyofaunal diversity using DNA barcoding as a molecular appliance both for marine as well as freshwater fishes. Khedkar et al. (2014) constructed firstly a DNA barcode library for freshwater fishes of Narmada River.They collected 820 fish specimens from different locations across the river basin. They generated a 314 different COI sequence and revealed that the specimens belonged to 85 species representing 63 genera, 34 families and 10 orders. Their study revealed that, out of 85 species, 5 species were endemic to India whereas 3 were exotic [21]. In addition, Lakra et al. (2011) barcoded the Indian marine fishes for the first time using mitochondrial COI gene. They characterized 115 marine fish species belonging to 7 orders and 37 families including Carangids, Clupeids, Scombrids, Groupers, Sciaenids, Silverbellies, Mullids, Polynemids and Silurids of Indian marine fishes for the generation of DNA barcodes. The average Kimura two-parameter (K2P) distances increased hierarchically from within species to orders [22]. Sachithanandam et al. (2012) carried out the identification of Epinephelus spp. in the Andaman coastal region by DNA barcoding using the

COI gene. They evaluated the mean genetic distances using Kimura 2 parameter (K2P) between the

studied Epinephelus spp. from Andaman coastal region and same species from the world over. Genetic divergences among Epinephelus longispinis, Epinephelus ongus and Epinephelus aerolatus were 0.0004, 0.0183, and 0.0437, respectively [23]. While, Sadurudeen et al. (2017) generated DNA barcodes for 32 fish species, delineating 13 families of order Perciformes in the Indian Coast [24].

**Regional Status and Scope of Study:**

No significant work has been done in Latur district upon genomic fish diversity by DNA Barcoding.

**Scope of study: present research work will useful for**

- Making Molecular Database for Future Researchers
- Study of new genomic variation affected by variable physicochemical parameter.

**Proposed work plan with Tentative Chapter Scheme**

**First Year:**

- **Chapter I**: Introduction.
- **Chapter II**: Collection of review of literature
- **Chapter III**: Study morphological fish diversity: Identification by Day Volume
- **Chapter IV:** Study of Physico-chemical parameter in relation to fish diversity

**Second Year:**

- **Chapter V:** Study of fish diversity population by Weiner index (H), Simpson Dominance index(D), Simpson index of diversity (1-D).
- **Chapter VI:** Study genomic fish diversity by DNA Barcoding

**Third Year:**

All statistical calculation and graph making, Thesis writing and submission process

**Future perspective:**

1. Identification of new species source
2. Making Molecular Database for Future Researchers

**Reference:**

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**Date:** 14/09/2022

**Place:** Latur

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